Conditioned Diuresis in Man: Effects of Altered Environment, Subjective State, and Conditioning Experience

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It has been possible to produce water diuresis in human subjects by the repetition of certain portions of a sequence which had repeatedly accompanied the ingestion of 750-cc. water loads. The environment used during conditioning was important for elicitation of the response. "Anticipatory" responses rarely occurred under conditions of regular reinforcement, but when the subjects became aroused during the experiments, or when schedules of reinforcement were intermittent, anticipatory responses occurred predictably. Simple conditioned responses were similarly augmented by these factors. Subjective perception of affective arousal was not necessary for augmented response magnitude to occur under the altered conditioning schedules.

Urinary composition was characteristic of reduced antidiuretic hormone levels. An hormonal response can thus be established in the human to previously neutral stimuli and appears subject to augmentation or inhibition by alterations in the subject's environment, his state of arousal, and the pattern of recent experimental routine.

THERE HAS BEEN a strong tendency in this country to regard the "classical" conditioned response as a physiological curiosity, or even artifact, bearing little or no relation to such important activities of higher organisms as the maintenance of homeostasis and directed behavior. The studies described below were carried out in humans and involve a neuroregulatory system long accepted as a model homeostatic mechanism: the neurohypophysis and its endocrine secretion, antidiuretic hormone (ADH), which plays a major role in the preservation of water balance. Observations were made regarding the interaction of the subjects' experiences and the performance of this physiological system after conditioning. Those experiences found to have important effects upon the magnitude of the diuretic response arose both from the subject's life outside the experimental laboratory as well as from

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the conduct of the conditioning procedure itself.

The clinical observations which led to the present study indicated that a diuresis of water and salt may occur as part of the response of some subjects to an apparently threatening situation.[1,2] In those studies only a small percentage of subjects tested lost sufficient water and electrolytes to alter fluid homeostasis appreciably and many showed little or no diuretic response. Although genetic factors may be invoked to explain this interindividual variability, it seemed more profitable to study the process by which an individual may *acquire* the potentiality of responding to a given experience by significant diuresis.

Studies published by others on the conditioning of diuresis have been limited to those using dogs and have emphasized the difficulties encountered.[3,4] In apparently successful studies the mechanism responsible for increased urine output has not been clear.[5,6] The problem involved in persuading dogs to drink large quantities of water over short periods of time led to the use of stomach or rectal intubation, a procedure which elicited emotional responses, usually accompanied by oliguria. Human subjects therefore offer certain practical experimental advantages over dogs. They also provide an opportunity to study those characteristics of conditioned responses which are uniquely human. A previous paper[7] has described in detail the methods used and the physiological evidence for concluding that decrease in posterior pituitary antidiuretic hormone output is the efferent mechanism for the diuresis.

The variability of the responses of a given individual was found to be even greater from one occasion to the next than the variation between individuals; a detailed report has been published elsewhere.[7] The present paper describes those factors which appeared to us most important in determining the variation in magnitude and timing of the response. In general, it became evident as we worked with conditioned humans that "arousal" greatly augmented the conditioned response; however, small changes in the conditioning routine from one day to the next had an equally great effect, even though this might not be associated with awareness of altered subjective state. Thus, this homeostatic system, through the process of conditioning, became involved in the pattern of the subject's everyday life, and the two, formerly quite independent, came to have important effects upon each other.

Experimental Methods and Procedures

The stimulus used in conditioning was the rapid oral ingestion of a moderate water load (750 cc.) by an adequately hydrated subject. It is generally held that the decrease in blood osmolality which follows the absorption of hypotonic fluid is detected by sensitive receptor sites in the anterior hypothalamus, and that secretion of antidiuretic hormone is thereupon inhibited. The renal tubules then respond to the lower circulating levels of this hormone by decreasing their reabsorption of water. A copious flow of dilute urine follows without altered rate of solute excretion, until the excess water is eliminated. No method exists for directly measuring small changes in the amount of antidiuretic hormone in the blood, but it is considered that these may be estimated from changes in urine flow and concentration, provided that excretory rates for electrolytes are constant and no gross changes in glomerular filtration rate occur.[8,9] This method of estimation was used in these experiments. Since the subjects were not catheterized, the completeness with which they emptied their bladders was checked by the measurement of their endogenous creatinine excretion. Catheterization was regarded as unnecessary since studies have shown that adequately hydrated male subjects can empty their bladders with satisfactory regularity at 20-min. intervals.[10,11]

The procedures followed by the 7 sub-

jects involved schedules of measured water intake, controlled diet and activity, and abstinence from smoking and alcohol during the entire experimental period. In addition, position and ambient temperature were controlled during the experiments themselves.

In order to create a standard base line of hydration, subjects were given an excess of water until urine flow exceeded 2 cc./min. The rate of ingestion was then reduced to 150 cc./hr. or stopped entirely and the subjects were studied during the subsequent period of declining urine output. Minute volume was measured over several control collection periods prior to water ingestion, as well as subsequent to conditioning stimuli.

After 3–7 control days during which only a single 30-cc. swallow was taken in the laboratory, all subjects were conditioned by receiving 750 cc. of water on repeated experimental sessions. In one experimental series the water was given immediately upon the subjects' entering the laboratory; for the other subjects, delays of 40 min. or 2 hr. were set to allow for anticipatory responses. The acquisition of a conditioned response could be demonstrated by giving a 30-cc. swallow of water instead of the customary 750 cc., and comparing the subsequent rise in urine flow (simple conditioned response) with flow rates observed on control days before conditioning. Under special conditions, the occurrence of a distinct rise in urine flow *before* water ingestion was noted (anticipatory response).

Urine was voided at 20- or 30-min. intervals into graduated cylinders for volume measurement, then frozen, and later analyzed for osmolality, sodium, potassium, creatinine, and chloride composition by standard techniques.[7]

Observations were made on the magnitude, timing, variability, and composition of anticipatory and simple conditioned responses, as well as on the effects of alterations in the environmental setting of the swallow and the state of hydration of the subject. Naturally occurring and drug-induced changes in the subject's state of arousal were observed as they affected magnitude of response. The term "arousal" is used in this paper to denote increased alertness and involvement, a change in subjective state away from drowsiness or apathy and largely independent of specific affect or mood. The subjects were interviewed briefly and informally at the beginning of each experiment and an effort was made to detect any significant changes in subjective state that day. The use of one of the investigators (M.H.) as subject for some of the experiments allowed a depth of introspection not possible for the other subjects under the conditions of the experiment. The possibility of unconscious distortion by the investigator-subject was minimized by the replication of all major findings in uninvolved subjects. Finally, variations in length and scheduling of conditioning experience were investigated as determinants of the magnitude and regularity of conditioned responses. A summary of the experiments conducted is given in Table 1.

Results

The Conditioned Responses

Altered response

The altered response to the swallow after 8–15 days conditioning with large water loads was intensively studied in 4 subjects. The three phases of the experiment for one of the supine subjects (B.B.) are illustrated in Fig. 1. The pattern of urine output following the 30-cc. swallow was appreciably altered by the subject's conditioning experience, so that diuresis was produced, mimicking the first 40 min. of the response to the 750-cc. water load used in conditioning. The 2-hr. delay before water ingestion allows the initial diuresis of recumbency[12] to subside. The changes in urine flow rates from control levels before the swallow to peak poststimulus rates in 18 experiments before, and 17 experiments after conditioning for the 4 subjects participating in this portion of the investigation are reported in detail elsewhere.[7] Urine flow rates before conditioning showed an inconsistent variability best ascribed to random factors (mean change, 0.13 cc./min., $p > .5$). How-

TABLE 1. SUMMARY OF EXPERIMENTS

*Category**	*Description**	*Subjects*	*No. of Expts.*	*Results*
Altered response to swallow	Response to 30 cc. before and after conditioning	K. K. S. R. B. B. S. B.	11 8 9 7	Mean Δ† = +1.38 cc./min. after conditioning (see table[7])
Anticipatory responses	Observations during initial regular conditioning procedure	B. B. S. B. M. H.	15 13 13	One clear-cut response (Fig. 2) No clear-cut response No clear-cut response
Nature of conditioning stimuli	Conditions of environment and time	K. K. S. R.	6 4	Responses absent outside lab. (see Table 2)
	Altered environment	K. K. S. R. M. H.	1 1 1	Δ = +0.1 cc./min. Δ = +0.6 cc./min. Δ = −0.7 cc./min.
	No swallow	K. K. M. H.	1 1	Δ = +0.2 cc./min. Δ = +0.9 cc./min.
Subjective state	Arousal during expt.	M. H.‡	1	Δ = +11.3 cc./min. (see Fig. 4)
	Arousal prior to expt.	B. B. M. H.	1 8	Δ = +4.8 cc./min. (see Fig. 5)
	10 mg. dextroamphetamine	B. B. M. H. B. B.	1 1 1	Δ = +4.7 cc./min. Δ = +7.8 cc./min. Δ = +1.6 cc./min.
Schedules of conditioning	Altered conditioning routine on previous day(s)	K. K.§ S. R.§ M. H. B. B.§ M. H.§	1 1 1 1 1	Δ = +2.1 cc./min. (after swallow) Δ = +1.5 cc./min. (after swallow) Δ = +6.6 cc./min. (after swallow) Δ = +4.5 cc./min. (anticipatory) Δ = +4.9 cc./min. (anticipatory)
	Extinction	K. K.	6	See Fig. 6
	After 6 days' reconditioning	S. B.	4	Δ (range) = −0.7 to +0.4 cc./min.
	Ratio reinforcement (1:3) (1:5) (1:7)	 M. H. J. M. G. H.	 15 15 13	Increased anticipatory responses. See Fig. 7

*See text.
†Δ refers to differences in minute volume: peak period minus control period.
‡Composition determined, part III of text and Fig. 4.
§Composition determined, part III of text and Fig 8.

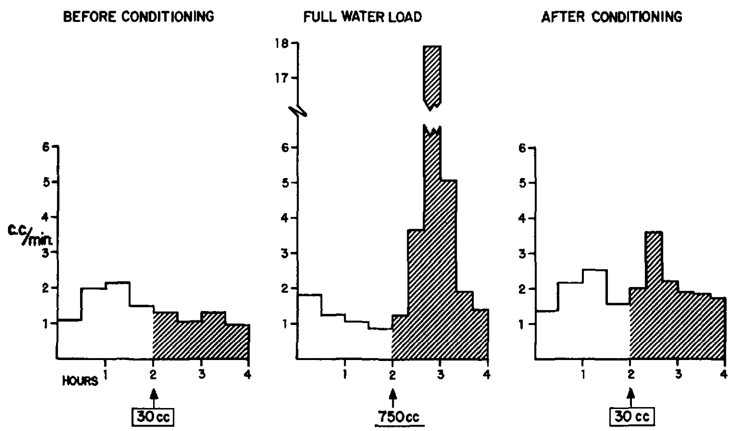


FIG. 1. Flow rates for three stages of conditioning in supine subject.

ever, after conditioning, an increase in urine flow followed stimulation by the swallow on all occasions, the mean increase of +1.38 cc./min. being significant ($p < .001$). Repeated presentation of the single swallow after conditioning led to gradual loss of the acquired response and a return to control values (see below).

In relatively dehydrated subjects with urine flow rates well below 1 cc./min. during control periods, the conditioned responses were greatly diminished (as were even the responses to full water loads). However, increased hydration did not augment diuretic responses beyond a 2–3 cc./min. increase above control flow rates, even with baseline rates of nearly 3 cc./min. These characteristics, as well as the chemical composition of the urine produced, are consistent with a neurogenic suppression of antidiuretic hormone as the physiological mechanism responsible for these conditioned diureses.[7]

Anticipatory responses

In the experiments thus far described, the single swallow of cold water served as a triggering stimulus for the conditioned subject, and in its absence, only insignificant increases in urine flow could be recorded under ordinary circumstances. However, in the case of the supine subjects for whom water ingestion was delayed after they entered the laboratory, urine flow was occasionally observed to increase in the collection period *before* water ingestion, apparently in anticipation of the impending water load and reminiscent of the salivation of Pavlov's dogs on merely being led into the experimental room. This phenomenon, as it occurred in one subject on the eleventh day of conditioning, is illustrated in Fig. 2. The sudden rise in urine output occurred 30 min. before water was given and, after water ingestion, continued to exceed the range observed for the other 14 days of this series by highly significant margins until the peak, when minute volume returned within the expected range.

The factors responsible for the sporadic occurrence of such anticipatory responses were not immediately clear. In addition it had been observed that the

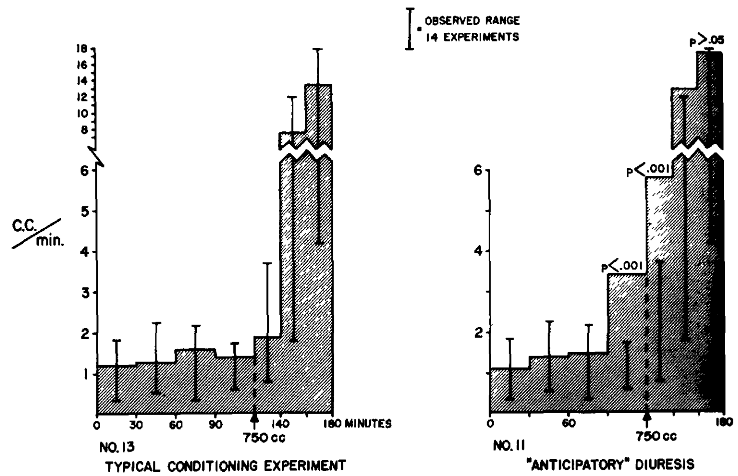


FIG. 2. Flow rates illustrating anticipatory diuresis in supine subject.

response to the swallow was far greater on some occasions after conditioning than on others.[7] Subsequent experiments were undertaken to identify some of the factors responsible for this variability of response.

Factors Modifying Conditioned Responses

Nature of conditioning stimuli

Since, during the conditioning, the act of coming to the laboratory and the sensory experience of being in the particular experimental room were regularly associated with the full water loads, the subject's environment would be expected to acquire importance in determining the diuretic response to the swallow. Indeed, for all subjects studied, it was found that the simple act of swallowing small amounts of water after conditioning was not in itself sufficient to cause diuresis. As illustrated in Fig. 3, the ingestion of as much as 150 cc. of water *outside* the laboratory in preparation for the experiment served merely to maintain control levels of urine flow, whereas a single 30-cc. swallow taken in the laboratory in which the subject had previously received 750 cc. on 23 occasions, elicited a marked increase in urine flow with an accompanying fall in osmolality. Table 2 contains data from the 10 experiments on 2 subjects after conditioning in which the effects of the 30-cc. swallow taken in the laboratory can be compared to the effect of 150 cc. taken an hour earlier in the subject's class or dormitory rooms under similar conditions of activity and position. The mean flow rates for the hour prior to the last ingestion of 150 cc. served as base line, to which is compared the mean of the highest flow rates of the two 30-min. collection periods following this 150-cc. drink. The mean change recorded was +0.23 cc./min., with $p > .4$ for this difference. However, 30 cc. taken in the environment which had in the past been repeatedly associated with the ingestion of large quantities of water, produced a mean rise of +1.37 cc./min. ($p < .005$) with-

in the next hour. No such diuresis followed 30-cc. swallows in experiments carried out in an identical fashion before conditioning (see above). Obviously a time discrimination is also operative, in that the subjects had previously been given the full 750-cc. load only at 5 P.M., and water swallowed prior to this time was never in excess of 150 cc. However, in three additional experiments after conditioning in which the 30-cc. swallow was given at the customary time of day (5 P.M.), but in a totally different environment (e.g. at home), little or no diuretic effect resulted (Table 1).

The anticipatory response described above (Fig. 2) indicated that the environment on occasion could itself promote diuresis before the triggering stimulation of the single swallow. However, it was apparent that proper circumstances must exist for this to occur predictably (see under *Schedules of Conditioning* below). When the swallow was omitted in testing after *regular* conditioning, the response observed in two subjects was only a slight phasic elevation and decline of baseline rates without the clear-cut diuretic peak apparently triggered when the subject was stimulated by the swallow.

Thus, under *ordinary* conditions the conditioned diuretic response could be demonstrated convincingly only when

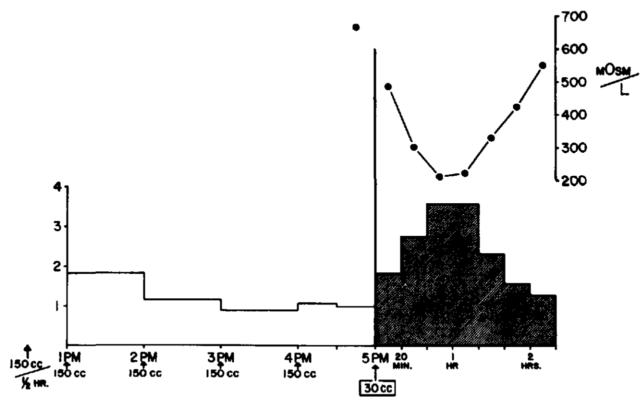


FIG. 3. Response (flow rates) to 30-cc. swallow after 23 conditioning days for a seated subject.

TABLE 2. EFFECTS OF ENVIRONMENT ON RESPONSES TO WATER INGESTION AFTER CONDITIONING*

	Mean values (cc./min.)†			
Water ingested	*Baseline*‡	*Maximum*‡	*Difference*	*p*§
150 cc. p.o. outside lab.	1.72 (1.6)	1.95 (1.2)	+0.23	>.4
30 cc. p.o. in lab.	1.52 (0.8)	2.89 (1.3)	+1.37	<.005

*Ten experiments.
†Figures in parentheses indicate standard deviation.
‡See text.
§Significance determined by t-test for nonindependent samples.

both the swallow and the conditioning room were presented together.

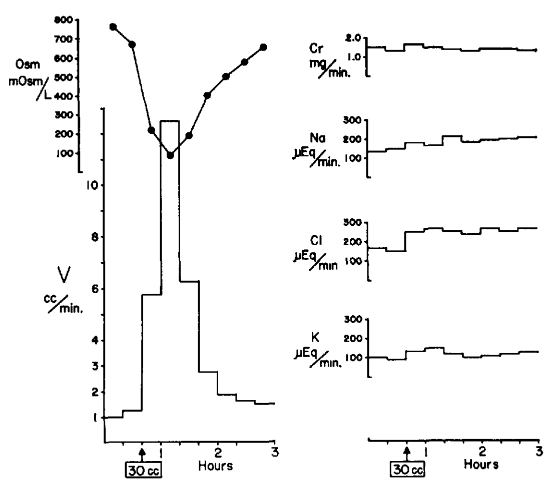
Subjective State of the Subject

It seemed to make very little difference whether the subject knew ahead of time that he would be given 30 cc. on a particular day instead of the usual 750 cc. Two of the subjects actually planned when they should receive the test swallow, and two others were kept completely uninformed until the water was presented. Since the diuresis followed the swallow by 40–60 min., all subjects were aware of the new circumstances at the time of response. Possibly, if the experimental design had allowed us to keep the amount of water intake unknown to the subject (e.g., by I. V. route of administration), larger responses might have occurred.

The simple diuretic response to the swallow was greatly augmented when the subjects became aroused, as evidenced by reports of being "excited" or "keyed up" and behavior consistent with this. Figure 4 illustrates the diuretic response obtained when the subject (one of the investigators, M.H.), who was personally involved in the design of the experiments, was anxious as to their outcome. As the desired effect was produced, he became elated; a peak flow of 12.4 cc./min. was recorded on that day. The pattern of this response may be compared to those occurring in an uninvolved subject (Fig. 1) and more nearly resembles the full response to 750 cc. than the usual conditioned response to 30 cc.

Similarly, the appearance of ***anticipatory*** diuretic responses seemed to be related to the occurrence of unusual increases in the subject's degree of arousal just prior to the experiment. Figure 5 presents the data from a longitudinal study of a single subject over 2–3 months' time. Standard conditions of hydration and sedentary activity were observed throughout. The subject (M.H.) had first been conditioned by receiving 750 cc. on 25 occasions; conditioned responses to the single swallow had been demonstrated, but no clear-cut anticipatory responses had occurred during

FIG. 4. Conditioned response (flow rates) in aroused subject (supine) with composition of diuresis.



schedules of regular reinforcement. Each day he entered the laboratory room at the same time (9:30 A.M.), lay down, and collected urine for two 20-min. collection periods. He then drank 750 cc. of water and remained for an additional hour. The volume of urine produced during this 40-min. anticipatory period on each experimental day is indicated in Fig. 5. No gross alterations in subjective state were reported by 5 non-conditioned subjects, similarly prepared and studied on 29 control days. For the 6 separate days shown under *A* (Fig. 5), the subject changed his usual morning routine, driving his 4-year-old son to school before coming to the laboratory. The subject described these, his son's first days at school, as "a big moment for me as well as for him. I was proud, excited and a little bit anxious about how it would all work out." Following his arrival at the laboratory on each of the next 6 days a striking anticipatory diuresis manifested itself with a crescendo rise in flow rates occurring after ½ hr. in the laboratory and reaching a peak at the customary time for water ingestion. The urine flow rate for the 30-min. collection period after arrival at the laboratory, but before entering the experimental room, was no different from the previous days, so that it did not appear that the subject's response to his son's first days in school was diuretic per se. Rather, the occurrence of an anticipatory diuresis on these days appeared to be an example of the effect of arousal on the timing and magnitude of the conditioned response. Subsequently, on returning to his ordinary morning routine, there was a return to baseline flow rates. At *B*, the subject had an interview in the half hour prior to the experiment, during which he was unexpectedly offered a position of new responsibility and experienced feelings of a conflicting nature. Five days later, at *C*, he received notification of an important success just prior to the experiment, and came to the laboratory elated. Finally, under *D*, he took 10 mg. of dextroamphetamine 1 hr. prior to the experiment. It produced a state of excitement and arousal, and anticipatory urine volume was augmented on this day, as on the days of naturally-occurring emotional arousal. This effect of dextroamphetamine was replicated in a second conditioned subject (Table 1); extensive experimentation with this drug has not

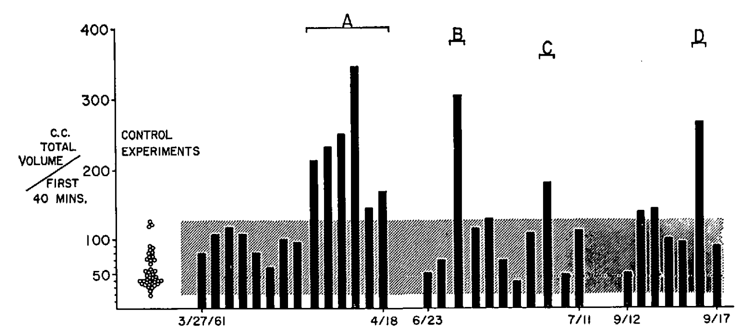


FIG. 5. Effects of increased arousal on anticipatory conditioned response magnitude of supine subject under regular reinforcement. Clusters of circles at left indicate values on 29 control days for 5 nonconditioned subjects similarly prepared and studied. Shading indicates the range (normal) for these subjects.

revealed similar responses in nonconditioned subjects.[13]

Conversely, the subjects also reported *decreases* in their arousal state, evidently as a result of the stereotyped, repetitive nature of the conditioning procedure. Indifference, apathy, and occasionally sleep were described by 3 of the 7 subjects (S.B., B.B., and M.H.) during one or two experiments after as little as a week to 10 days of conditioning. It appeared to us that conditioned responses were more difficult to demonstrate on those days. However, this particular relation between regular schedules of conditioning, decreased arousal state, and diminished response magnitude was exceptional, and reduced conditioned diuresis was often observed in patients perceiving no drowsiness or lethargy after long periods of regular reinforcement with full water loads.

Schedules of Conditioning

It was the usual observation that alterations in conditioning procedure were followed by augmentation of conditioned response magnitude, but with minimal or no change in reported arousal state.

It was possible to observe in 6 subjects the effects of omission of the usual water load (unconditioned stimulus) and early termination of the experiment on one or two experimental days. Such disturbances of routine were often followed by augmented responses on subsequent days (Table 1). When the timing afforded a delay before water ingestion, anticipatory responses as high as 11.2 cc./min. were recorded in supine subjects, and the response to the 30-cc. swallow, superimposed on the anticipatory response, rose as high as 7.8 cc./min. in a seated subject, or 3–4 times maximum values obtained after regular conditioning. The subjects reported no difference in subjective state on these days, but it seems possible that an imperceptible degree of uncertainty and consequent alertness was produced by the altered routine, even in experienced subjects.

Thus, maximum conditioned responses did not follow directly after periods of regular conditioning routine, but could be brought out by important alterations of the conditioning sequence for 1 or 2 days. Figure 6 shows the responses ob-

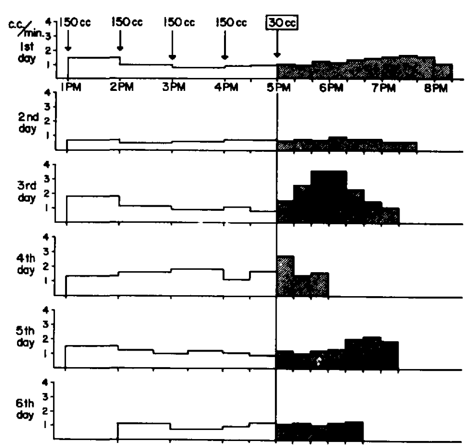


FIG. 6. Consecutive daily responses (flow rates) to 30-cc. swallow after 23 conditioning days for seated subject.

served on 6 sequential days in which the swallow was presented alone after 23 days of regular conditioning. As was observed in 3 of the 4 subjects so tested, it was not the first trial of the 30-cc. swallow after conditioning which gave the most striking diuretic response. There appeared to be a latent period of one or two trials before a clear-cut diuresis occurred. (The response on Day 2 may have been inhibited also by relative dehydration on that day.) Further repetition of the swallow thereafter caused it gradually to lose its acquired diuretic effect, with a return to control levels (extinction, Day 6). An unusual pattern is observed on Day 1 and Day 5, with urine flow rising only late in these experimental sessions after more than an hour's delay following the swallow. A similar effect was observed in the other subject extensively studied in this manner. This urine flow pattern, observed both on the first trial after long periods of conditioning and, later, in extinction, suggests a similar inhibitory process at work on both occasions.

Further information suggesting that conditioning can be easily overdone was provided by one subject (Table 1) who gave two clear-cut diuretic responses on his first two trials after 13 days of conditioning, but after having been given 6 additional days with large water loads he failed entirely to show diuresis in response to the swallow, despite repeated trials.

Thus, physiological habituation seemed to develop very readily in these human subjects under stereotyped repetitive conditions, sometimes, but not always, in association with feelings of apathy and indifference. The decreased physiological responsiveness characteristic of this habituation, could be dispelled by alterations in experimental routine for 1 or 2 days. The response to the 30-cc. swallow was then augmented, and if the conditioning had been carried out with delay prior to water ingestion, anticipatory responses occurred.

These findings suggested that anticipatory conditioned responses might be regularly observed if reinforcement with large water loads was given intermittently rather than on a regular schedule. To test this hypothesis, 3 subjects on standard schedules of hydration were studied over 2–3 months. After the subjects entered the laboratory and lay down, a delay of 40 min. was set before water ingestion, during which time anticipatory diuresis might be observed. Means and standard errors of urine volumes produced during this anticipatory period under different reinforcement ratios for the 3 subjects are shown in Fig. 7. All subjects were studied first for 15–25 days under a one-to-one reinforcement ratio; that is, they were given 750 cc. of water every time they came to the laboratory. Under this schedule, none of the subjects showed any trend in their urine output from day to day, although sporadic anticipatory responses did occur in 2 of the subjects. Then, ratios of reinforcement were changed for a period of 13 or 15 days, one subject receiving water on the average of once every third experimental day, the second every fifth day, and the third subject every seventh day. S-1 knew in advance of these changes, but S-2 and S-3 did not. The results support the hypothesis: these periods of intermittent reinforcement were associated with a sustained increase in the anticipatory urine output in all 3 subjects, with a confidence level of 1–2% for the differences between means.

Finally, all subjects were returned to the one-to-one ratio, and this significantly inhibited anticipatory diuresis in S-1, but did not reliably do so within eight trials in S-2 and S-3. Further experiments would be required to ascertain whether this differential sensitivity to inhibition was determined by the size of the ratios used during intermittent rein-

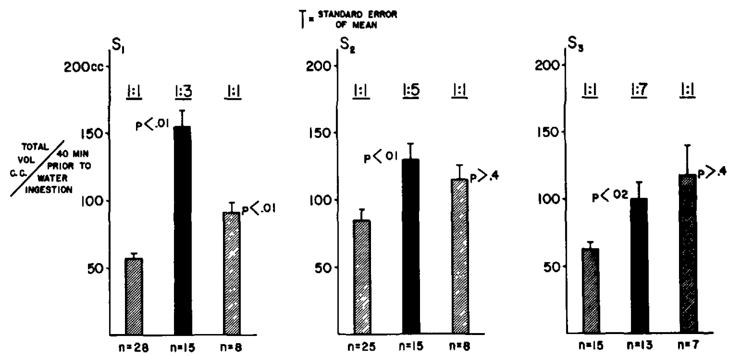


FIG. 7. Ratios of reinforcement as determinant of magnitude of anticipatory diuresis, means, and standard errors of means.

forcement, the fact that only S-1 was informed of the plan of the experiment, or by other interindividual differences.

Composition of Augmented Conditioned Responses

A previous paper has described the composition of the simple conditioned response to the 30-cc. swallow after regular conditioning.[7] The pattern consisted of the addition of considerable quantities of free water to the urine without alteration in regulatory mechanisms affecting excretion rates of electrolytes or glomerular filtration rate (as estimated by creatinine excretion), a pattern specific for decreased circulating ADH.

The augmented responses described in this paper were of identical qualitative pattern, the higher flow rates being the product of increased dilution, with excretion rates for electrolytes and creatinine no different from those for control days. Figure 4 shows the data from one of the experiments associated with arousal;* a similar pattern was found in the experiment in which dextroamphetamine had a like effect on this subject.

The same qualitative pattern is evident in the composition of the augmented diuretic responses following altered schedules of conditioning. Figure 8 shows the mean changes for minute volume, osmolality, and electrolyte and creatinine excretion rates for 4 subjects before conditioning and compares these with those changes observed after altered conditioning schedules in the same subjects. Of the latter group, two of the responses were anticipatory in timing and two followed stimulation by the 30-cc. swallow (Table 1). In the experiments after conditioning, "change" for a given variable was calculated by subtracting the value measured in the initial collection period from the value found in the collection period of peak diuresis. For each subject, identically timed collection periods from the experiments *before* conditioning were utilized in the same way so that valid comparisons could be made between the two

*The slowly rising rates of sodium and chloride excretion evident in Fig. 4 are characteristic for the few hours of recumbency,[15] while the small increase in all excretion rates in the third collection period is to be expected as a result of washout of renal dead space during the precipitous rise in flow rates at this point in the experiment.

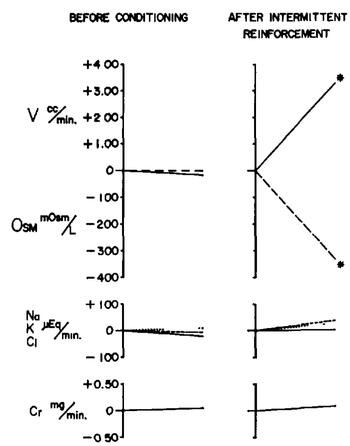


FIG. 8. Mean changes in urinary composition during experiments before conditioning and after altered schedules of conditioning for 4 subjects. Terminations of lines indicate changes in values from initial collection period to period of peak diuresis. Long-dashed line indicates osmolality; short-dashed line, Na; dotted line, K; and asterisk, $p < .02$.

series. Values for the initial collection period in the experiments after conditioning were not found to be significantly different from those before conditioning ($p > .2$ in all cases), so that the subjects appear to have been in similar states of water and electrolyte balance on the two occasions.

No significant changes in urinary composition were observed in the experiments before conditioning. After altered schedules of conditioning, the increase in minute volume was accompanied by a commensurate fall in osmolar concentration. Excretion rates for electrolytes and creatinine remained relatively stable during the diuresis, the small changes which occurred being similar to those observed on control days. Using the t-test for nonindependent data,* the only two significant changes were in osmolality ($p < .02$) and minute volume ($p < .01$) in the experiments after conditioning. All other changes were insignificant ($p > .1$).

One can conclude, from the stability of creatinine excretion, that no major change occurred in glomerular filtration rate. Likewise, there is no evidence of activation of regulatory mechanisms affecting sodium, potassium or chloride excretion. The addition of considerable quantities of free water to the urine can be ascribed to decreased circulating ADH[7, 9] which, in turn, may be inferred to have taken place in response to the previously neutral conditioning stimuli.

The diuresis previously reported with emotional arousal[1, 2] has usually involved considerable increases in electrolyte excretion and glomerular filtration rate, so that the conditioning experience would appear to have modified the responses to arousal observed in these subjects. However, it seems possible that with more threatening events, other regulatory mechanisms might be superimposed.

The evidence is consistent with the hypothesis that a common effector mechanism exists for the simple conditioned response to a swallow after regular conditioning, the augmented response of heightened arousal, and the anticipatory response after altered schedules of conditioning.

Discussion

Physiological regulatory mechanisms are generally understood to serve homeostasis by their ability to adjust to disruptions of the *milieu interieur* as these are

*Because of the small N, a standard t-test was also applied. This test supported the conclusions above at the level $p < .005$ for the differences in osmolality and minute volume after conditioning. All other differences were insignificant ($p > .3$).

imposed by the environment. In this way, the neurohypophysis is sensitive to the dilution of blood following large water loads and allows the excess water to escape reabsorption by the renal tubules so that it appears in the urine. The conditioned response would appear to be a still more subtle capability of this neuroregulatory system, allowing the adequately hydrated organism to prepare for the absorption of a large quantity of water by an increased urine flow *before* the water is actually ingested, thus minimizing the disturbance to homeostasis. However, the complex nature of this physiological refinement and its dependency on past conditioning experience rather than present homeostatic demands renders the system liable to responses which are inappropriate in timing, magnitude, or both.

Conditioning provides the organism with a potential for maladaptive responses, but it is not certain what role, if any, conditioning may play in the development of disease. However, the results of the present study indicate first of all, that the human can acquire specific physiologic responses to signal stimuli within relatively short periods of conditioning. Secondly, conditioned subjects show impressive augmentation of these newly acquired responses when they are aroused, as well as during intermittent conditions of reinforcement which approximate more closely the haphazard conditions of everyday life. This evidence points to ways in which specific conditioning experience might determine the pattern of psychophysiologic reactions encountered clinically.

In addition, the present findings have a practical application in suggesting an explanation for the observations of laboratory investigators in the fluid and electrolyte field who have previously reported as artifacts instances in which human subjects have responded specifically to certain cues in the experimental procedure as if the physiologic stimulus had been administered.[16, 17] From the results of the present study, it would appear that conditioning may complicate any series of repeated experiments on a single individual which involve stimulation of neuroregulatory mechanisms.

To summarize, the conditioned responses described in this paper represent an important and hitherto unrecognized regulatory capability of the neurohypophysial system. The method provides a useful experimental model for the study of psychophysiologic responses in the human. Several of the observed characteristics suggest that conditioning could play a role in the genesis or modification of clinically important disease processes. Finally, laboratory experimentation may become confounded by inadvertent and unrecognized conditioned responses.

A few observations made in these experiments are of interest in the light of current conditioning literature. The influence of the conditioning environment and the importance to the response of the whole sequence of behavior leading up to each experimental session supports the concept of "afferent synthesis" so elegantly elaborated from neurophysiological studies by P. K. Anohkin.[18] Other recent Russian work on the use of interoceptive stimuli in conditioning[19] has indicated that stimulation of gut such as occurred with the swallow of cold water in our experiments, may be effective as a conditional stimulus, even without conscious perception. In humans, although more slowly etsablished, conditional responses to interoceptive stimuli are unusually resistant to inhibition based on conscious "set" and to experimental extinction. Possibly, they are integrated more completely at a lower level in the central nervous system.

Several unexpected findings deserve additional comment. The observed increase in conditioned response magnitude with the first few trials of extinction

violates the classic pattern of simple exponential decrement established by Pavlov for salivary and gastric secretory conditioning in the dog. However, extinction patterns similar to those encountered in our experiments have been reported in human eyelid,[20] vascular,[21] and GSR[22] conditioning, as well as in conditioned EEG alpha-wave block.[23] Those studies, like the present one, involved "defensive" unconditioned stimuli (air jet, cold, and bright light), all apparently of biological meaning different from Pavlov's feeding of the hungry dog. However, recent Russian work describes increasing responsiveness ("disinhibition") during early extinction after delayed salivary conditioning in the dog.[24] In order to explain the diminished responsiveness found on the first one or two trials after regular conditioning, one must consider the phenomenon of "habituation," currently of great interest to neurophysiologists[25] and first described in connection with conditioning by Pavlov.[14] He found that many dogs, after months of alimentary conditioning with the same stimuli, ceased to respond to conditional, and finally, even to unconditional stimuli. Only by radical change of routine could responses be restored. Evidence from more recent studies suggests that the concentration of conditioning stimulation in a short period of time is responsible for this inhibitory effect,[26] but, in the present studies, it was evident even with 24 hr. elapsing between reinforcements.

It may be postulated that, in a laboratory setting, the human is unusually susceptible to habituation (possibly a physiologic equivalent of boredom or apathy). While he is also susceptible to conditioning, a dynamic equilibrium is set up during repeated association in which the tendency for the organism to respond to cues as if they were the associated physiologic stimulus is counterbalanced by the tendency to diminish responsiveness progressively to repeated stimuli of the same nature (habituation). One might refer to mechanisms for "positive" and "negative" learning, both of vital use to the organism but in different situations. In all systems studied, habituation is dispelled quite rapidly by change in the stimulus pattern,[27] whereas conditioned responses are more resistant.

Thus, the subjects may have failed to manifest a conditioned response to the first swallow after conditioning because of interference by habituation or "inhibition of reinforcement" created during repetitive stimulation. However, the change of stimulus routine from full water load to single swallow for 1-2 trials dispelled habituation, allowing the positive conditioned response to become evident on the third trial (Fig. 6). The same factors help explain the appearance of *anticipatory* conditioned responses on the occasion after the subjects had been twice allowed to alter routine by leaving the room without drinking. The successful maintenance of anticipatory conditioned responses during intermittent reinforcement (Fig. 7) may also be a result of such variable conditions preventing inhibition by habituation. These findings suggest that schedules of reinforcement, so important to operant conditioning, should also be considered as a determinant in classical conditioning.

Under these altered schedules of reinforcement, the subjects were not usually aware of any changes in their arousal state. These findings provide evidence that variations in experience can, under certain circumstances, produce specific physiologic change, *independent* of the individual's subjective reaction to the situation. A similar process may be involved in certain clinical examples of apparent dissociation between physiologic and "emotional" responses of the human to his life situation. Yet it should be emphasized that before conditioning can be invoked to explain clinical phenomena, clear-cut evidence must be

gathered that repetitive associative experience involving a particular organ system has actually taken place in the past life of the patients. This has not yet been done, and the relevance of conditioning to psychosomatic disease processes must remain speculative, albeit intriguing.

Summary

In order to investigate the adaptive behavior of a single, neurohumoral, regulatory system in the normal human, 7 subjects on controlled diet, hydration, and activity were conditioned by receiving large water loads in the same laboratory room each day for at least 2 weeks. After their conditioning, a single 30-cc. swallow taken in this setting produced a significant diuresis in all subjects, whereas 150 cc. taken in a different setting but under otherwise similar conditions, merely maintained control flow rates. "Anticipatory" increases in urine flow, occurring just prior to water ingestion, were also observed during the early stages of conditioning.

The magnitude of conditioned responses was increased during coincident states of heightened arousal and minimized when the subjects were apathetic or drowsy. A state of adequate hydration was also found necessary for demonstration of response.

The regular conditioning procedure led to diminished magnitude of response when continued for more than 15–20 sessions, and conversely, schedules of intermittent reinforcement led to augmented responses and the appearance of anticipatory responses. However, appreciable changes in subjective state were not usually perceived by the subjects on these occasions.

Analysis of urinary composition during these altered conditions showed marked increases in free-water clearance without alteration of excretory rates for electrolytes or creatinine—a pattern characteristic of decrease in circulating antidiuretic hormone.

These experiments indicate that a vital neurohumoral regulatory mechanism can participate in a learning process, and by this means is capable of responding in terms of past experience, even in preference to current homeostatic demands. The method provides an experimental model for the study of psychophysiologic reactions in the human.

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The Academy of Psychoanalysis will hold its two-day Annual Meeting at the Hotel Biltmore, Los Angeles, Calif., on May 2 and 3, 1964. Inquiries may be addressed to Alfred H. Rifkin, M.D., Secretary, The Academy of Psychoanalysis, 125 East 65 St., New York 21, N. Y.